



Tim Donohue University of Wisconsin-Madison GLBRC Director Professor of Bacteriology

Fuel Synthesis & Sustainability Activities

Biomass 2009 March 17, 2009







GLBRC Partners

Academic

UW-Madison
Michigan State University
Illinois State University
Iowa State University
DOE National Labs
Pacific Northwest NL
Oak Ridge NL
Industry
Lucigen/C5-6 Technologies

DOE Office of Science

Joint Genome Institute
BACTER Program
Advanced Scientific Computing

States of Wisconsin & Michigan

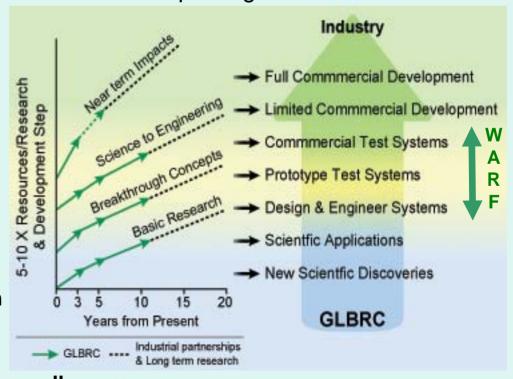
Facilities (WI) & faculty (WI/MI)

Technology Transfer

WARF, others

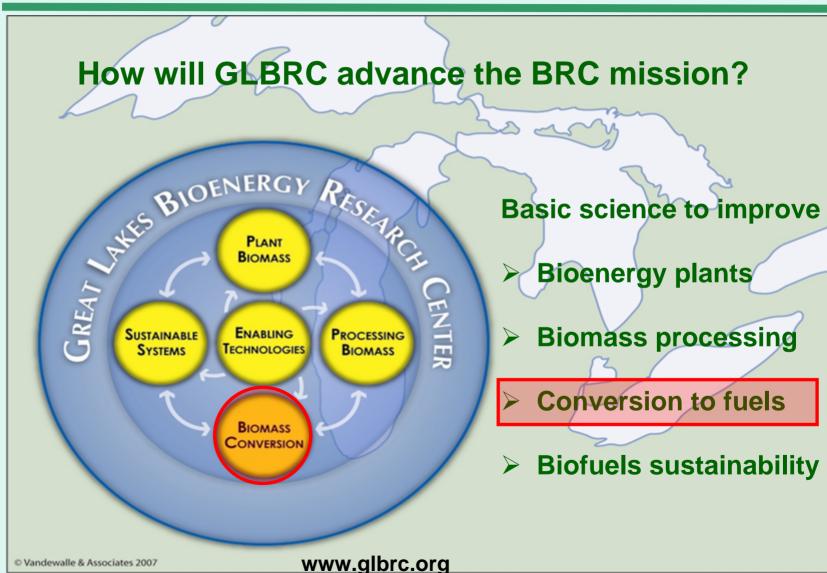
Advancing the Cellulosic Biofuels Mission

- genome-enabled analysis of model systems & bioenergy organisms/ecosystems
- scientific underpinnings of tomorrow's biofuels













Biomass conversion into energy products: improve methods for converting plant biomass into materials that can replace fossil fuels.

- Microbial catalysts optimized for fuel synthesis
 - production of lignocellulolytic enzymes
 - efficient use of 5- & 6-carbon sugar monomers & oligomers
 - ethanol and alternative fuel synthesis (biodiesel & alkanes)
 - stress, fuel & lignotoxin tolerance

Photosynthetic microbes optimized for

- H₂ production
 - CO₂ sequestration into fuels

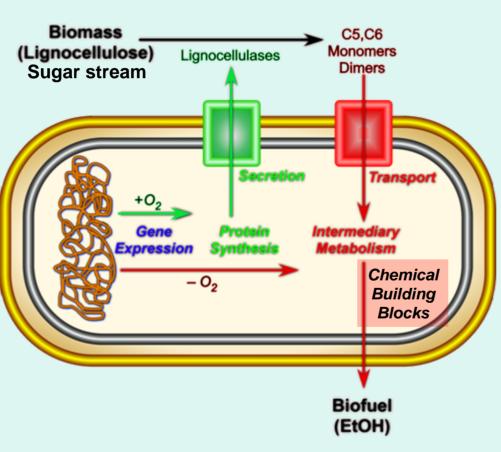
Chemical catalysts optimized for

- dehydration of sugars to furfurals and derivatives
 - hydrogenation of furfurals and derivatives to high-energy fuels





Improved Microbial Fuel Production



Phase 1: Aerobic growth, secrete degradative enzymes

Phase 2: Anaerobic growth (*fermentation*), activate enzymes & transporters to produce fuels/building blocks

Optimize sugar stream refining

- > transport sugar mixtures
- > produce fuels & building blocks
- > resist cellulosic & fuel toxins





REDIME: REiterative Directed MIcrobial Evolution

combines synthetic biology, computational & natural strain optimization

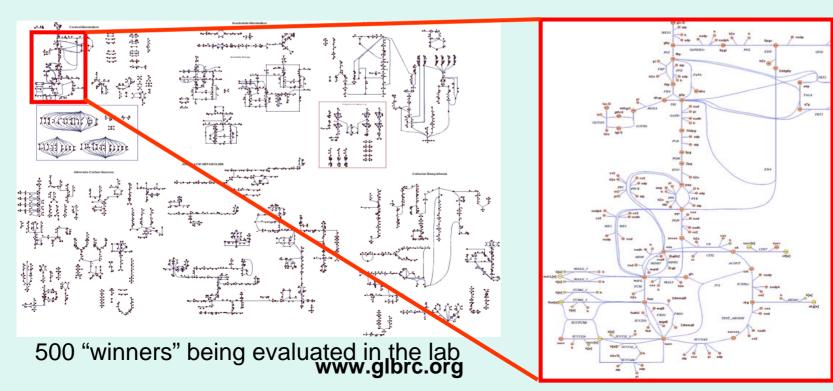
Strain Engineering	\rightarrow	Strain Optimization
Rational engineering	Microbial	Microbial directed evolution
Metabolic engineering, Expression engineering, Genome optimization,	candidates (many)	Conventional selections (e.g., toxin resistance), experimental evolution
Comparative genomics, Protein engineering		High-throughput phenotype screening
Stochastic strain variation		Biofuel (<i>e.g.</i> , ethanol) production, Lignocellulase production, Fuel and Lignotoxin tolerance
Targeted or Global mutagenesis, Metagenomics, Network rewiring		
A		Microbial
Targets (bottlenecks) iden- tified for reengineering		candidates (few)
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tified for reengineering Computational Modeling		(few)
Computational Modeling Data-driven comprehensive models of the microbal state		(few) Experimental Fermentation
Computational Modeling Data-driven comprehensive models	Multiomic data	(few) Experimental Fermentation Variation of conditions Biomass source, pH, Temperature,





Optknock Modeling of Ethanologenesis

- ➤ Includes 904 *E. coli* metabolic genes (~20%), 931 reactions & 625 metabolites (updated from Reed et al. Genome Biology. 2003)
- ➤ Based on Ingram et al. strains, predict growth rates & fuel yields in combinations of single, double (6 x 10⁴) & triple (7 x 10⁷) knockouts







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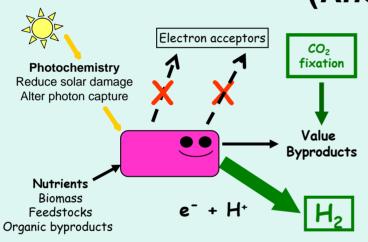
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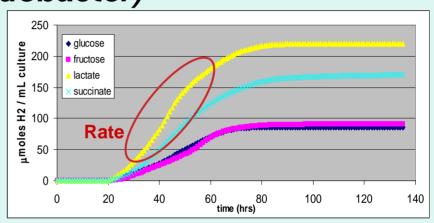
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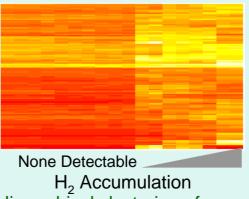


Solar- & biomass-powered fuel production (Rhodobacter)

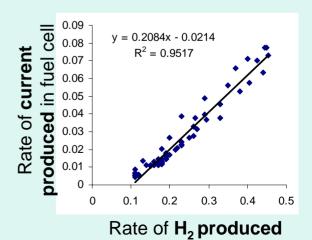








H₂ Accumulation Hierarchical clustering of gene expression as H₂ accumulates







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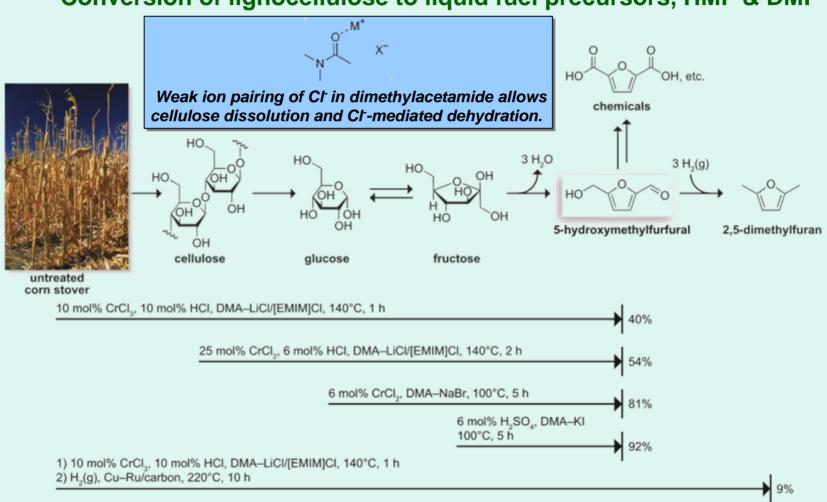
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Great Lakes Bioenergy Research Center



Conversion of lignocellulose to liquid fuel precursors, HMF & DMF

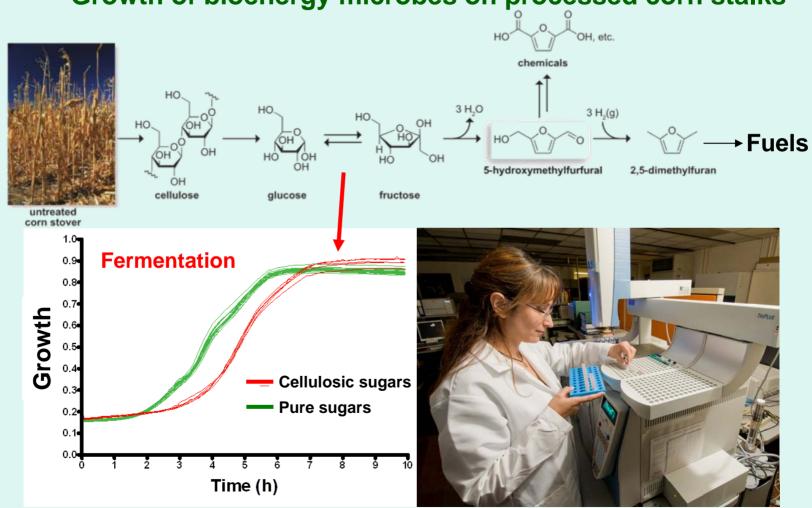


Binder, J. B & R. T. Raines 2009 JACS 131:1979-1985 **www.glbrc.org**





Growth of bioenergy microbes on processed corn stalks

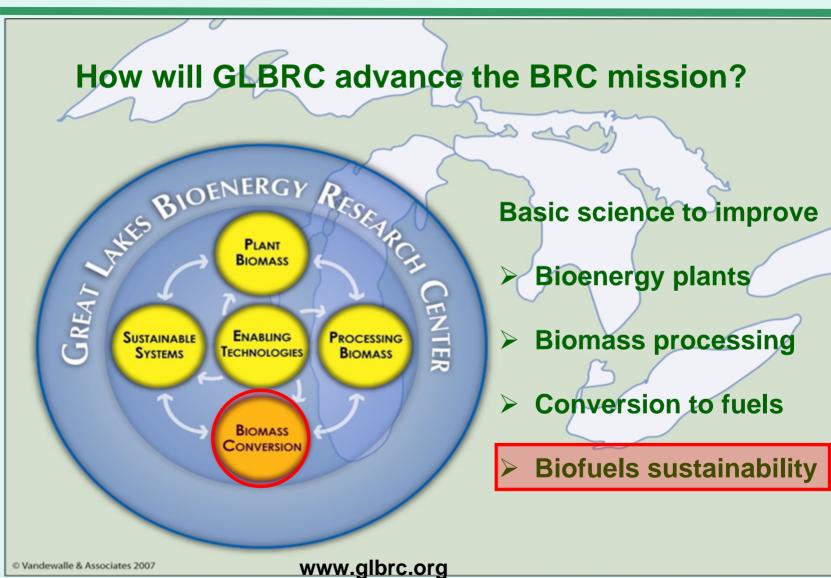


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Vandewalle & Associates 2007









Improved Bioenergy Sustainability

High Input, Low Diversity (annuals)

Develop economically viable & environmentally responsive ecological, agricultural & life cycle practices (WI & MI Agricultural Stations)

Continuous Corn Corn-Soybean-Canola







Low Input, High Diversity (perennials)

Native prairie Early successional



Overcome bottlenecks in agricultural, industrial, & behavioral systems to

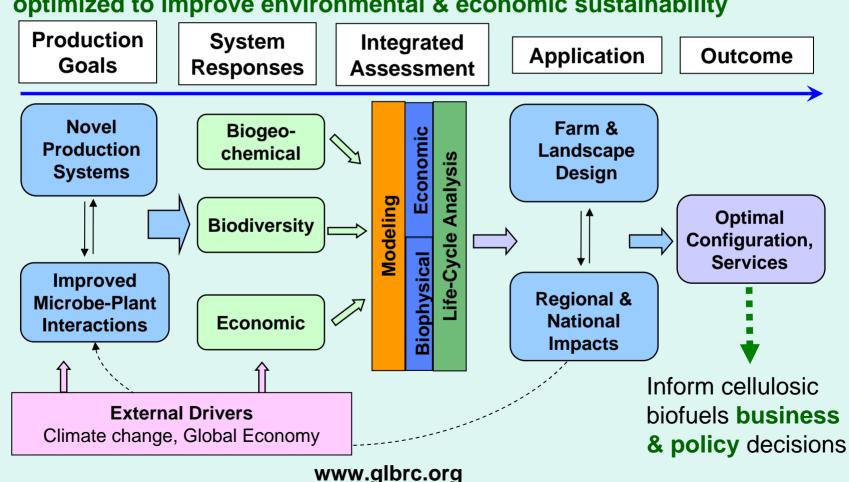
- Improve carbon neutrality & greenhouse gas mitigation across the entire biofuel life cycle at multiple scales
- Improve ecosystem services in biofuel landscapes (e.g. water, soil & air quality, biodiversity, pest suppression, land use) www.glbrc.org





Sustainability Research Goals

> Predict elements of integrated biofuel production systems that can be optimized to improve environmental & economic sustainability







Cellulosic Biofuels:

An obvious new venue for the Wisconsin Idea

